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A new pygmy leafmining moth, *Stigmella tatrica* sp. n., from the alpine zone of the Tatra Mountains (Lepidoptera, Nepticulidae)

Zdenko Tokár[1], Aleš Laštůvka[2], Erik J. van Nieukerken[3]

1 *P. J. Šafárika 11, SK-92700 Šaľa, Slovakia; zdeno.tokar@gmail.com*

2 *Slavíčkova 15, CZ-796 01 Prostějov, Czech Republic; aleslastuvkaento@seznam.cz*

3 *Naturalis Biodiversity Center, PO Box 9517, NL-2300 RA Leiden, The Netherlands; nieukerken@naturalis.nl*

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Abstract. *Stigmella tatrica* sp. n. is described from moths taken in the alpine zone of the Tatra Mountains in Slovakia. The new species is similar to several other montane species of *Stigmella* Schrank, 1802 in the *S. aurella* (Fabricius, 1775) group in external characters and male genitalia; its closest relative is *S. dryadella* (O. Hofmann, 1868). It is indistinguishable from *S. tormentillella* (Herrich-Schäffer, 1860) by the colour and pattern elements of the forewing; however, it differs in the male genitalia in the shape and number of cornuti, in the female genitalia by long apophyses with protruding ovipositor, and by COI barcodes. Immature stages are unknown, but *Dryas octopetala* L. (Rosaceae) is the possible hostplant. The long ovipositor suggests an unusual, possibly hidden place for oviposition. The male and female adults and genitalia of both sexes are figured and photographs of the habitat are provided.

Introduction

The pygmy leafmining moths or Nepticulidae have around 300 species in Europe, the great majority of which are leafminers, feeding on woody plants and they are abundant in forested areas (van Nieukerken et al. 2016). Overall they do not form an important aspect of the diversity of high mountain faunas (but see Stonis et al. (2016) for a group of *Stigmella* Schrank, 1802 species occurring in the high Andes). In Europe few Nepticulidae are adapted to high altitudes, and apart from some common species with large altitudinal ranges, only a few species of *Stigmella* are alpine specialists, particularly a group of species feeding on herbaceous Rosaceae (Klimesch 1981; Johansson and Nielsen 1990; Laštůvka and Laštůvka 1997).

The high altitude *Stigmella* species of the Tatra Mountains, especially above the tree line, have been poorly studied and all records reside in a handful of publications. In the Polish part of the alpine zone *Stigmella* species were first observed by Toll (1948) and Borkowski (1970). Buszko et al. (2000) mentioned three *Stigmella* species from the alpine grasslands: *S. pretiosa* (Heinemann, 1862), *S. aeneofasciella* (Herrich-Schäffer, 1855), and *S. dryadella* (O. Hofmann, 1868). From the Slovak part of the Tatras, only one faunal contribution is known: Gregor (1986) found mines of

S. dryadella in mid-October in the Belianske Tatra Mountains, Kvetnica and Bujačí Vrch Mountain at altitudes between 1500 and 1900 m (the number of mines and hostplant were not given). Most European alpine Nepticulidae belong to the *Stigmella aurella* (Fabricius, 1775) group, a Holarctic group of 18 named species (12 in Europe), all but one of which feed on Rosaceae, particularly herbs and shrubs such as *Rubus*.

In late July 2005, while inventorying the Lepidoptera fauna of the Western Tatra Mountains, the first author with his friend found four males of an unknown *Stigmella* species in alpine grasslands at altitudes between 1800 and 1900 m. Detailed study of two specimens by the second author was inconclusive, because of the relatively high similarities of their external appearance and male genitalia to closely related *Stigmella* species in the *S. aurella* group.

Later on, additional males were found in the eastern part of the Tatras, Belianske Tatra Mts at altitudes of ca. 2000 m. These specimens were sent to the third author for DNA analysis. In 2016 the first author's last visit to the locality brought success in the form of a large series of specimens, including two females. Individuals of the new species were observed flying around the low cover of high-altitude plant communities with *Dryas octopetala* L. An examination of the female genitalia of this tiny moth, as well as the analysis of the DNA barcodes, showed that it represents a hitherto undescribed *Stigmella* species.

Material and methods

All specimens were taken as adults during daylight, either using a net or caught directly from the leaves of *Dryas octopetala* into small glass vials. The genitalia were dissected in the usual way for small Lepidoptera, the preparations being stored in glycerol in small plastic vials or embedded in Euparal on glass. The drawings of the male and female were made by the second author using water colours. Drawings of the genitalia were made by the first author using Indian ink on transparent sheets. Photographs of the type locality were taken using digital cameras Canon PowerShot G11 and Nikon Coolpix P600.

For methodology of DNA barcodes we refer to papers of the third author and his colleagues (van Nieukerken et al. 2012a; Doorenweerd et al. 2015). The specimen data for barcoded specimens are given in the public BOLD dataset DS-STIGTATR. Many of these barcodes were earlier published by van Nieukerken et al. (2012b). For the alpine species *Stigmella stelviana* (Weber, 1938) and *S. geimontani* (Klimesch, 1940) we were only able to obtain short barcodes of 146 base pairs. Tree-building was performed with PAUP and Phylip plugins in Geneious R8.1.8, as outgroup we used a specimen of *Stigmella tityrella* (Stainton, 1854).

The nomenclature of species follows the "Revised classification and catalogue of global Nepticulidae and Opostegidae" (van Nieukerken et al. 2016).

Abbreviations

AL	Aleš Laštůvka
BOLD	Barcode of Life Database
Gp	Genitalia preparation
RMNH	Naturalis Biodiversity Center, Zoological collections, Leiden, The Netherlands
ZT	Zdenko Tokár

Taxonomy

Stigmella tatrica Tokár, Laštůvka & van Nieukerken, sp. n.

http://zoobank.org/97DD8700-29A6-4628-B331-8EE0ADC5E965

Material. Holotype: ♂, pinned, with genitalia in glycerol in a small plastic vial. Original labels: "Slovakia, Belianske Tatry, Zadné Jatky, 1950–2010 m, 49°14.18'N; 20°13.50'E, 30.vii.2016, Zdenko Tokár leg.", "HOLOTYPE *Stigmella tatrica* Tokár, Laštůvka & van Nieukerken" (red label), coll. Z. Tokár (to be deposited in the Central Slovakia Museum Banská Bystrica).

Paratypes: 14♂, 2♀, same locality and data as holotype, Gp. ZT ♂ 12873–5, 12878, 12918, ♀ 12876, ZT leg., coll. ZT & AL; 1♂, František Kuraj leg. & coll.; same locality as holotype, 2.viii.2014, 2♂, Gp. ZT 12266–7, ZT leg., coll. RMNH, DNA samples Tokar 12266, 12267; Belianske Tatry, Bujačí Vrch, 49°13.48'N; 20°15.55'E, 4.viii.2011, 2♂, Ignác Richter leg. & coll.; Západné Tatry, Červené Vrchy, Stoly-Temniak, 49°13.28'N; 19°54.19'E, 29.vii.2005, 3♂, Gp. ZT 9085, 9087, 9132, ZT leg., coll. AL, DNA samples RMNH.5012163, RMNH.5012164 (DNA extracts in RMNH); 1♂, Gp. Richter 9847, Ignác Richter leg. & coll. All paratypes with red label "PARATYPE *Stigmella tatrica* Tokár, Laštůvka & van Nieukerken".

Description. Adult, male (Fig. 1). Wingspan 4.0–5.0 mm. Head: frontal tuft black, collar with black lamellar scales. Antenna black, with 32–35 segments. Scape white. Thorax and tegula glossy black. Ground colour of forewing brown with dark golden sheen; dark violet metallic reflection around postmedial fascia and towards the forewing apex. Fascia shining silver, wider at costal margin. Fringe grey. Hindwing grey, no androconial scales. Abdomen brown to black with whitish scales on margins of segment and whitish anal tufts.

Female (Fig. 2). Wingspan 4.0–4.5 mm. Antenna shorter, with 26 segments. Forewing markings as male. Abdomen with remarkable protruding ovipositor, no anal tufts.

Figure 1. *Stigmella tatrica* sp. n., male, holotype, wingspan 4.0 mm.

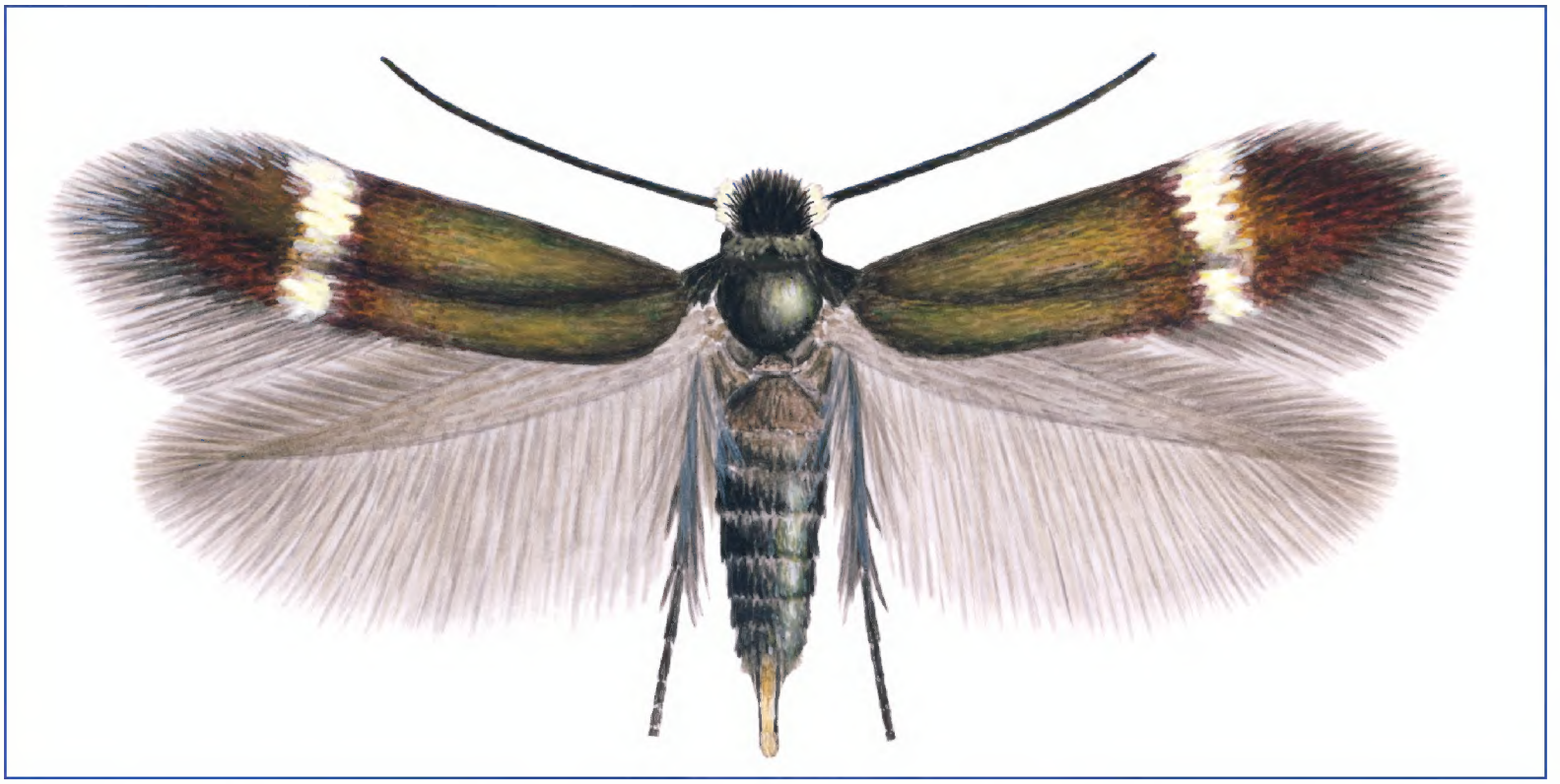
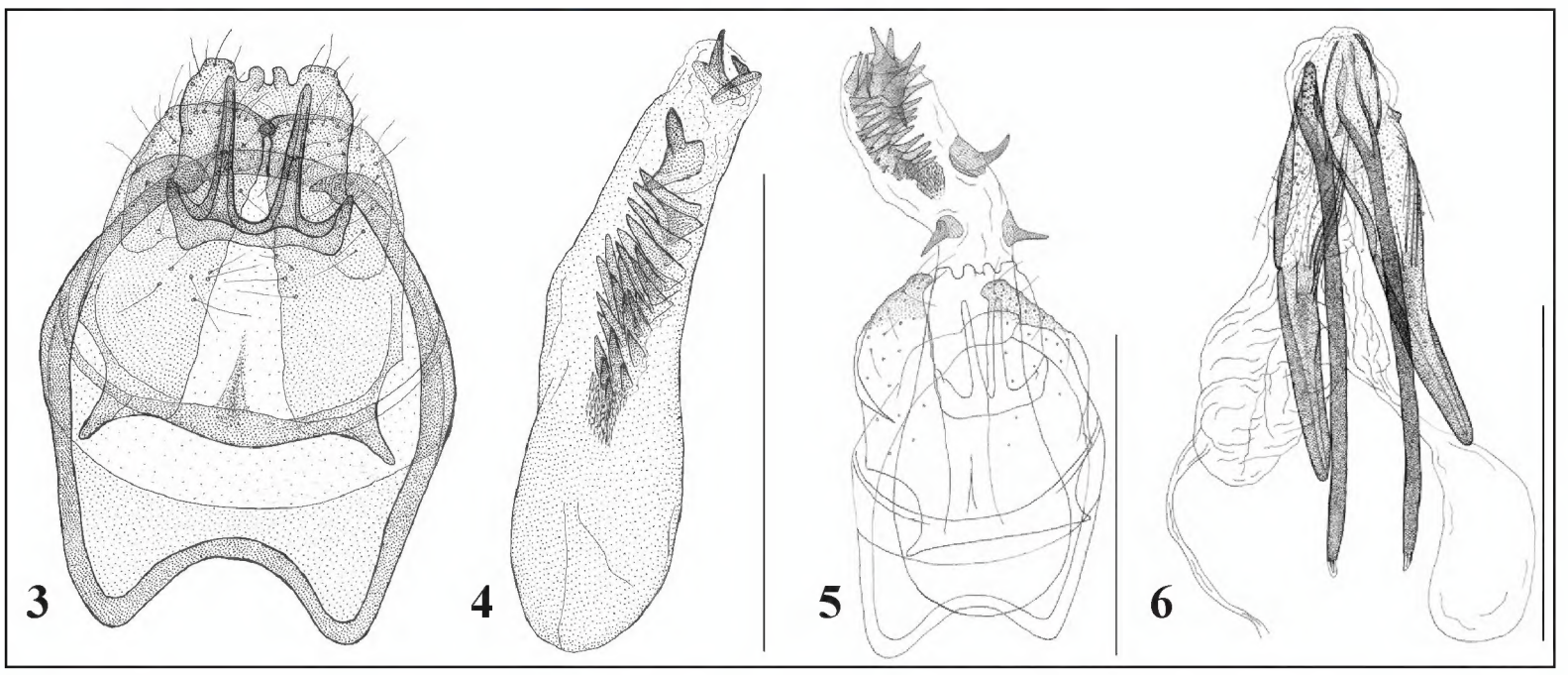


Figure 2. *Stigmella tatrica* sp. n., female, paratype, Belianske Tatra Mts, Zadné Jatky Mt., 30.vii.2016, wingspan 4.5 mm.



Figures 3–6. Male and female genitalia of *Stigmella tatrica* sp. n., paratypes. **3.** Male genitalia, Gp. ZT ♂ 9085, dorsal view; **4.** phallus; **5.** Gp. ZT ♂ 9087, vesica everted. **6.** Female genitalia, Gp. ZT ♀ 12876. (Figs 3–5, scale bar 0.25 mm; Fig. 6, scale bar 0.5 mm).

Male genitalia (Figs 3–5). Vinculum moderately long, anteriorly slightly bilobed. Uncus very broad, with posterior processes well separated, each bilobed. Gnathos with long and separated posterior processes. Valva rectangular with very short and blunt distal process. Transtilla broad with relatively short sublateral processes. Phallus cylindrical, slightly longer than genital capsule, vesica with long series of pointed cornuti of different sizes and three separate thorn-shaped cornuti near phallotrema.

Female genitalia (Fig. 6). Apophyses very long and strongly sclerotized, forming a conspicuous ovipositor protruding from abdomen. Posterior apophyses about 1.3 times as long as anterior apophy-

Figure 7. The Western Tatra Mts, Red Mts, Stoly Rocks, 29.vii.2005, locality where the first adults of *Stigmella tatrica* were obtained. Photograph Z. Tokár.

ses. Anterior apophyses very broad, well sclerotized anteriorly. Length of corpus bursae shorter than posterior apophyses, devoid of pectinations. Accessory sac heavily folded, longer than corpus bursae.

Diagnosis. *Stigmella tatrica* is somewhat similar to several other montane species in the *Stigmella aurella* group in forewing colour and pattern and in male genitalia. Externally it is indistinguishable from *S. tormentillella* (Herrich-Schäffer, 1860). Carpathian *S. dryadella* differs from the new species by more glossy forewings, a wider violet area before fascia and paler head and collar. In the male genitalia the new species is most similar to *S. geimontani*, *S. stelviana* and *S. aeneofasciella*, but it differs significantly from these externally. Unlike *S. tatrica*, *S. tormentillella* and *S. dryadella* have only one large cornutus in their vesica. In the female genitalia *S. tatrica* differs considerably from other species in the group by the long and strong apophyses, forming a protruding ovipositor.

Biology. The early stages of the new species are unknown. The adults were observed flying over low cover of high-altitude plant communities with the presence of *Dryas octopetala* and resting or quickly moving on leaves of that plant. Adults were collected during daylight hours between 29th July and 4th August (light collecting was not attempted). This likely represents a single annual generation.

Distribution and habitat (Figs 7–10). *Stigmella tatrica* is so far known only from the alpine zone of the Eastern and Western Tatra Mountains. In the Eastern Tatra it was found in the Belianske Tatra Mountains (*Belianske Tatry*), Zadné Jatky Mountain and Bujačí Vrch Mountain at an altitude of 1800–2010 m. In the Western Tatra it was taken in the Red Mountains (Červené Vrchy), Stoly Rocks at Temniak Mountain, near the Slovak-Polish border at an altitude of about 1800–1900 m.

Figure 8. The Belianske Tatra Mts, Zadné Jatky Mt., 30.vii.2016, type locality of *Stigmella tatrica*. Photograph Z. Tokár.

Figure 9. The Belianske Tatra Mts, Zadné Jatky Mt., 30.vii.2016, adult of *Stigmella tatrica* sitting on *Carex firma*. Photograph F. Kuraj.

Figure 10. The Belianske Tatra Mts, Zadné Jatky Mt., 30.vii.2016, adult of *Stigmella tatrica* sitting on *Dryas octopetala* leaf. Photograph F. Kuraj.

The Belianske Tatra Mountains and the Red Mountains are both karst areas, built of limestone and dolomite with a dominance of subalpine or alpine grasslands with many different plants growing there, many of which are endemic, rare or endangered species. The vegetation at the type locality could belong to the *Caricion firmae* Gams association, with the following higher plant species commonly present: *Carex firma* Host (Cyperaceae), *Dryas octopetala* L. (Rosaceae), *Androsace lactea* L., *Primula auricula* L., *Soldanella carpatica* Vierh. (Primulaceae), *Arenaria tenella* Kit., *Dianthus nitidus* Waldst. & Kit., *Silene acaulis* (L.) Jacq. (Caryophyllaceae), *Bartsia alpina* L., *Pedicularis oederi* Vahl. ex Hornem., *P. verticillata* L. (Orobanchaceae), *Bellidiastrum michelii* Cass., *Crepis jacquinii* Tausch (Asteraceae), *Bistorta vivipara* (L.) Gray (Polygonaceae), *Campanula cochleariifolia* Lam. (Campanulaceae), *Chamorchis alpina* (L.) Rich. (Orchidaceae), *Festuca versicolor* Tausch (Poaceae), *Galium anisophyllon* Vill. (Rubiaceae), *Pinguicula alpina* L. (Lentibulariaceae), *Ranunculus alpestris* L. (Ranunculaceae), *Saxifraga aizoides* L., *S. caesia* L., *S. paniculata* Mill. (Saxifragaceae), *Selaginella selaginoides* (L.) P. Beauv. ex Schrank et Mart. (Selaginellaceae); the following mosses: *Ctenidium molluscum* (Hedw.) (Schimp.) (Hylocomiaceae), *Ditrichum flexicaule* (Schwaegr.) Hampe (Ditrichaceae), *Tortella tortuosa* (Hedw.) Limpr. (Pottiaceae); and the following lichen: *Cetraria islandica* (L.) Ach. (Parmeliaceae) (Šibík et al. 2004; Kliment et al. 2010).

Etymology. The specific name *tatrica*, an adjective, is derived from the Tatra Mts, where the new species was discovered.

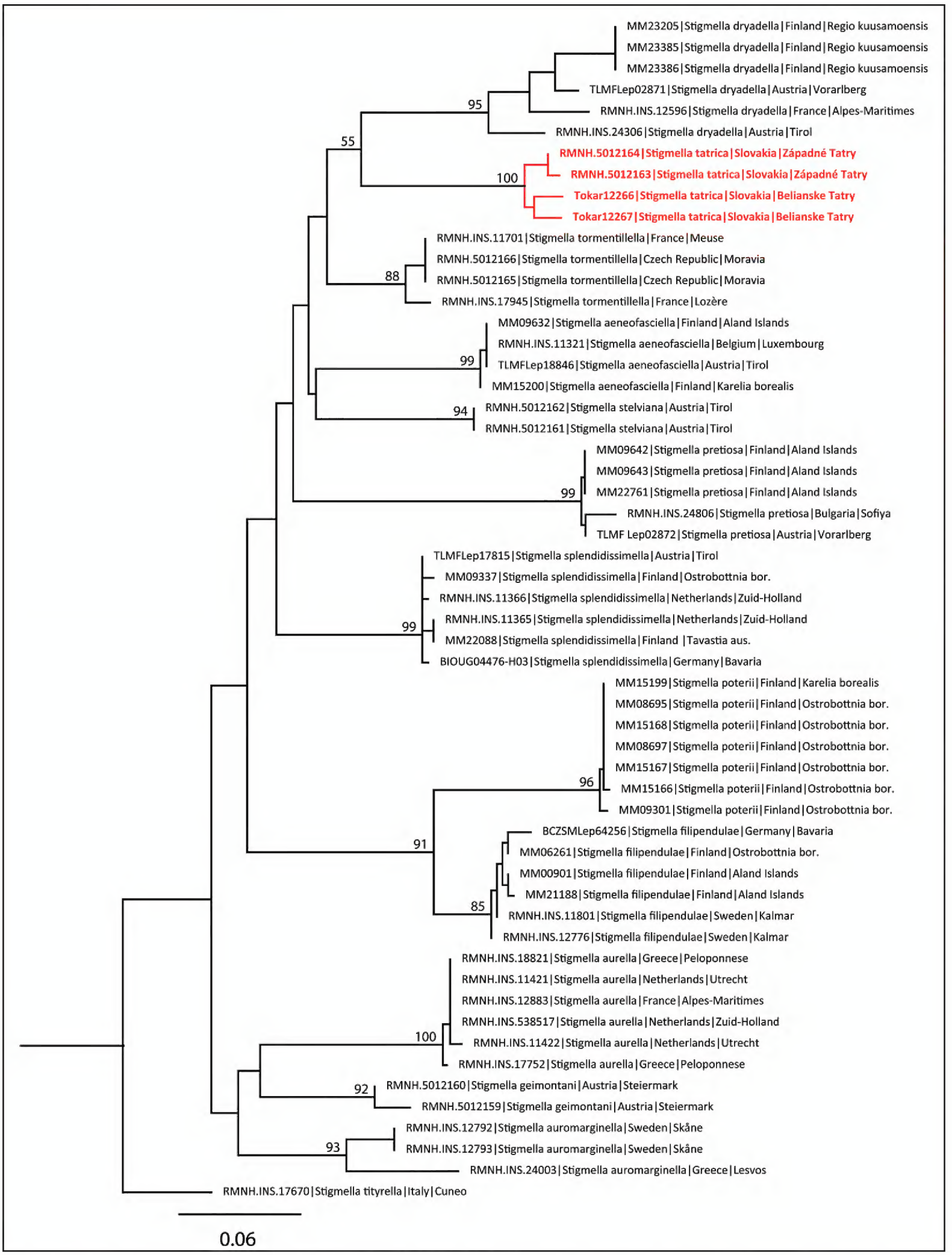


Figure 11. Maximum likelihood tree of European species of the *Stigmella aurella* group, showing the position of *Stigmella tatrica* in red. Small figures represent bootstrap values, after 100 replicates. Bootstrap values below 50 are not given. A specimen of *Stigmella tityrella* is used as outgroup. Nomenclature follows van Nieukerken et al. (2016).

Molecular data (Fig. 11). We obtained COI barcodes from legs of four specimens, two of which were partial barcodes. The Barcode Index Number is BOLD:ACU7181. The maximum K2P intraspecific distance for a full barcode is 1.5%. The nearest neighbour is *Stigmella tormentillella* at a distance between 6.2 and 7.1%. Both in Neighbour-Joining and Maximum Likelihood analyses *S. tatrica* groups consistently with *S. dryadella*, but bootstrap support is lacking.

Remarks

Borkowski (1970) described *Stigmella geimontani tatrensis* from the Polish Tatra Mts, reared from *Geum montanum* L. (Rosaceae). Later he synonymised it with *S. pretiosa* (see Borkowski 1975). In the paper from 1970 he recorded also *S. stelviana* (as ssp. *crantziella*) and *S. dryadella* from the Tatras. He found mines of *S. stelviana* on *Potentilla crantzii* Crantz & Fritsch (Rosaceae) from the end of August to mid of September at altitudes 1200–1800 m but no adult was reared from these. The mines could therefore also have been made by larvae of *S. tormentillella* that are feeding on various *Potentilla* spp. He also mentioned mines of *S. dryadella* on *Dryas octopetala.* They were observed in September and in May at an altitude of about 1800 m. The aforementioned record by Gregor (1986) of *S. dryadella* mines was also not supported by reared adults.

Discussion

The discovery of *Stigmella tatrica* shows that the montane fauna of Central European Nepticulidae is still insufficiently studied and can provide surprises. We expect that *S. tatrica* can also be found in the Polish part of the Tatra and other parts of the Carpathians, e.g. in Romania; currently it seems to be an endemic species for the Carpathians. Although the partial mitochondrial gene COI as used for DNA barcodes is usually not sufficient for a robust phylogenetic analysis, both analytical methods invariably group *S. tatrica* with *S. dryadella*, and this together with the fact that *S. tatrica* has usually been collected on or near *Dryas octopetala*, suggest that the two species are sister taxa whose ancestor shifted to *Dryas*. The protruding ovipositor suggests that *S. tatrica* has an unusual, possibly hidden oviposition site, and potentially rather different leafmines compared to *S. dryadella*, or even a different feeding mode.

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